Reviewed By: Irving Mauer, Ph.D., Geneticist 10-23-90
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 10/23/90
Toxicology Branch I - IRS (H7509C)

DATA EVALUATION RECORD 008139

I. SUMMARY

MRID (Acc.) NO.: 412552-24
ID No.: 7078-RT
RD Record No.: 253,112
Caswell No.: 623C (129017)
Project No.: 0-0339

Study Type: Mutagenicity - Sister-chromatid exchanges in vitro (CHO/SCE)

Chemical: CIDEX OPA Antimicrobial (ortho-phthalaldehyde)

Sponsor: Surgikos, Inc., Arlington, TX

Testing Facility: Microbiological Associates (M/A)
Bethesda, MD

Title of Report: Sister-Chromatid Exchange Assay in Chinese Hamster Ovary Cells.

Authors: D.L. Putnam and M.M. Morris

Study Number: (M/A) T8241.334

Date of Issue: October 28, 1988

TB Conclusions:

Dose-related positive for induced sister-chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells at 0.4 ug/mL and above under both activated and nonactivated conditions up to a toxic dose of 3 ug/mL.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - 913-12 (o-phthalaldehyde, OPA)

Description: Light yellow crystalline solid
Batch (Lot): 861-15
Purity (%): 99.7
Solvent/Carrier/Diluent: Distilled water (DW)

B. Test Organism - Mammalian cells (in vitro)

Species: Chinese hamster (ovary)
Strain: CHO (K_1)
Source: American Type Culture Collection (CCL #61), Rockville, MD

C. Study Design (Protocol) - This study was designed to assess the potential of OPA to induce SCE when administered in vitro to CHO cells according to an enclosed protocol based upon recognized (published) procedures and methods.*

A Statement of Quality Assurance measures (inspections/audits) was provided, as well as a statement of adherence to Good Laboratory Practice.

D. Procedures/Methods of Analysis - Following a preliminary dose-selection toxicity test (9 doses of OPA ranging from 0.05 to 500 ug/mL), duplicate cultures of CHO cells were exposed to five concentrations of test article, for 26 hours in the absence (-S9) of metabolic activation, but only for 2 hours in the presence of rat liver S9 (microsomal enzymes) from pretreated (Aroclor 1254) Sprague-Dawley males, followed by reculturing in nontest fresh medium for the balance of the 24-hour exposure period. Two hours after initiation of exposure, both sets of cultures were also treated with the nitrogeneous base analog bromdeoxyuridine (BrdUrd, 0.01 mM). The mitotic-arresting alkaloid, Colcemid was added to all cultures for the last 2 hours of incubation (final concentration, 0.1 ug/mL). In addition to DW (solvent) and untreated controls, other cultures were treated

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*Abe, S. and M. Sasaki (1977) Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Nat'l. Cancer Inst. 58: 1635-1641.
Latt, S.A., J. Able, S.E. Bloom, A. Carrano, E. Falke, D. Kram, E. Schneider, R. Schreck, R. Tice, B. Whitfield, and S. Wolff (1981) Sister chromatid exchanges: A report of the Gene-Tox Program. Mut. Research 87: 17-62.

similarly with the mutagens triethylmelamine (TEM 0.025 ug/mL) and cyclophosphamide (CP, 2.5 ug/mL), serving as positive controls for the nonactivated and S9-supplemented series.

At harvest, metaphase cells were collected by centrifugation and prepared for microscopic examination by conventional cytological methods on standard glass slides. Dried, fixed microscopic slides were then treated according to published procedures modified from the fluorescence-plus-Giemsa (FPG) technique of Perry and Wolff* (namely, staining in Hoechst 33258, exposure to ultraviolet light, and counterstaining with standard Giemsa).

Cells with a modal chromosome number (20±2) on coded slides (25 per culture; 50 per treatment) were scored for SCE in second division (M2+) metaphases, and cell cycle kinetics (percentage of cells in M1, M2, and M3) also recorded.

Only one complete trial of OPA was conducted. For this lab to consider an assay valid, the mean SCE/cell in negative controls must not exceed 16, while the value for the positive control must be twice (or greater) the negative control. A substance is considered positive if it induces twice (or greater) as many SCEs as solvent control at at least two consecutive (nontoxic) doses, statistically significant for these test doses according to Dunnett's t-test. A statistically significant increase at one dose and/or no dose-response renders the result equivocal, or negative according to the magnitude of response and/or the number of dose levels affected.

E. Results - In the preliminary toxicity test, growth inhibition as evidenced by changes in mitotic index (MI = cells in mitosis/500 cells counted) and lengthening of cell cycle traverse were used to select doses for the main assay (Report Tables 1 and 2). Based on 50 percent inhibition of M1 and severe cell cycle delay (close to 100% of cells still in M1) at 5 ug/mL OPA in both activated and nonactivated cultures, but respectable activity below this level (at 1.5 ug/mL, 65 to 75% M2 cells), 3 ug/mL was selected as the HDT for the main assay, plus a sequence of four half-doses below that, namely: 0.2, 0.4, 0.8, and 1.5 ug/mL ±S9.

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*Perry P. and S. Wolff (1974) New Giemsa method for differential staining of sister chromatids, Nature 251: 156-158.

Under nonactivated conditions, 26 hours treatment with OPA significantly increased the frequency of SCEs in a dose-responsive manner over solvent (= 12.56 SCE/cell) at 0.8 ug/mL (= 14.54 SCE/cell, $p < 0.05$) and 1.5 ug/mL (18.00 SCE/cell, $p < 0.01$) but was toxic (no M2 cells available for analysis) at the HDT, 3 ug/mL (Report Table 3, attached to this DER). In activated (S9-supplemented) cultures, 2-hour exposure increased SCE frequencies ($p < 0.01$) over solvent control (= 11.32 SCE/cell), in a dose-responsive manner at all subtoxic doses tested that could be evaluated, namely 0.4 ug/mL (= 13.48 SCE/cell) 0.8 ug/mL (= 15.20 SCE/cell), and 1.5 ug/mL (= 17.02 SCE/cell); the HDT, 3 ug/mL, again caused too much delay for analysis (Report Table 4, attached).

Both mutagens (TEM and CP) induced highly elevated levels of SCE (Tables 3 and 4).

The authors concluded that OPA induced increased SCE in CHO cells under both activated and nonactivated conditions, and is thus positive for this test system.

F. TB Evaluation - ACCEPTABLE. Although only one complete assay was conducted, this study was performed with appropriate controls and adequate procedures such that the data generated can only be interpreted as a definitive positive.

Attachments (Data Tables)

ATTACHMENT I

Report Data Tables

Page _____ is not included in this copy.

Pages 6 through 7 are not included in this copy.

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